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APPLICATIONS OF HYDROLYTIC AND DECARBOXYLATING ENZYMES IN BIOTRANSFORMATIONS.

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Peptides, and oligosaccharides and glycosides, can be synthesised by making use of the 'reverse hydrolytic activity' of proteases and glycosidases respectively. In applying these enzymes to the practical synthesis of these classes of compound, several factors need to be considered, namely the need to shift the rate-determining step through the use of activated substrates, the need to minimise competing hydrolysis of these and the need to minimise hydrolysis of the products. In spite of these problems, the enzymatic methods have many attractive features, not least amongst which is the absolute control of stereochemistry in acyl transfer and glycosyl transfer respectively.

Enzymes (lyases) that normally catalyse the cleavage of carbon-carbon bonds have been found to catalyse also their formation by 'abnormal' pathways. These enzymes are pyruvate decarboxylase (EC 4.1.1.1) and acetolactate decarboxylase (EC 4.1.1.5). A third enzyme, acetolactate synthase (EC 4.1.3.18), that catalyses carbon-carbon bond formation in the pathway of biosynthesis of the branched-chain amino acids, has a limited substrate range but its mode of action is of interest as it is homologous with pyruvate decarboxylase. This observation sheds light on the 'abnormal' reaction catalysed by pyruvate decarboxylase.

APPLICATIONS OF HYDROLYTIC ENZYMES

Introduction

In considering the mechanisms of action of proteases and glycosidases, a simplified scheme can be considered that embodies many of the important features of the mode of action of these classes of enzyme (Fig. 1). Binding of substrate to enzyme gives an enzyme-substrate complex ES that is converted into an acyl enzyme intermediate E.I by proteases, or into a glycosyl-enzyme intermediate by glycosidases. This intermediate, in the normal course of enzyme action is

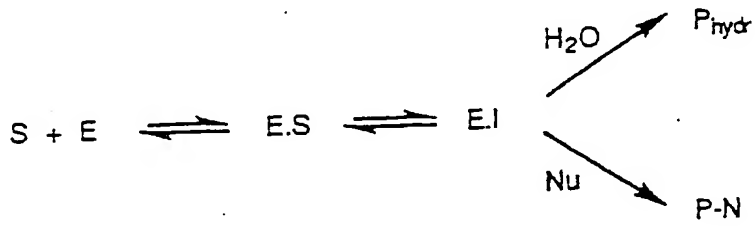


Figure 1. Simplified kinetic scheme for the hydrolysis of peptides and glycosides by proteases and glycosidases respectively. S = substrate, E = enzyme, E.S = enzyme –substrate complex, E.I = enzyme–intermediate complex, Nu = nucleophilic acceptor of peptidyl or glycosyl transfer. P_{hydr} = hydrolysis product; P-N = transfer product.

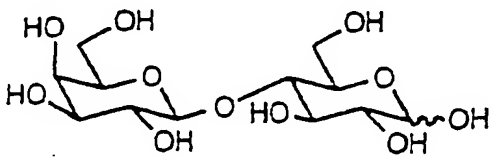
intercepted by water to give products of hydrolysis. The value of these systems in biotransformations stems from the observation that the intermediates can be intercepted not only by nucleophilic species N that resemble closely the normal leaving group generated on formation of the acyl-enzyme or glycosyl-enzyme intermediates, but also by nucleophiles which frequently markedly differ from these in structure.

When activated substrates are used, the rate-determining step can switch from capture of the intermediate E.I to its formation. This is the effect observed on

changing from peptide substrates of proteases to acyl amino acid esters, or from oligosaccharide substrates of glycosidases to nitrophenyl glycosides. In the case of glycosidases, this can result in an apparent 'activation' of the enzyme, resulting from rapid formation of the glycosyl-enzyme intermediate followed by rate-determining capture by a nucleophile other than water. The principles embodied in these kinetic considerations can be applied to practical biocatalysis, as will be seen below.

Glycosidase-catalysed Carbohydrate Synthesis

The impetus to develop new methods of carbohydrate synthesis has sprung from the recognition, rapidly increasing over recent years, that carbohydrates play a key role in many important molecular recognition phenomena (Ciba Foundation, 1989; Fukuda, 1992). Synthetic methods in carbohydrate chemistry have developed enormously in recent years, but construction of a target oligosaccharide is still a lengthy and costly exercise. Consequently, any method that offers to increase the efficiency of carbohydrate synthesis is of interest. The glycosidase approach (Nilsson, 1991) has the advantage that no protection-deprotection steps are involved and there is complete control of the configuration at the newly generated anomeric centre. Simples glycosides are readily prepared. Some recent examples from our laboratory are shown in Scheme 1. β-Galactosides were obtained using β-galactosidase as catalyst and lactose **1** as galactosyl donor. Although yields are low, the simplicity and low cost of the process neverthless make it a useful method. The yields in the examples of Scheme 1 decrease as the solubility of the acceptor alcohol decreases, limiting the concentration achievable and consequently the ability of the acceptor to compete with water. By contrast, with a water-soluble diol such as 1,3-butanediol, much higher yields are obtainable (Scheme 2) (Crout, MacManus and Critchley, 1991). Yields are based on donor as the acceptor is present in excess. The results described in Scheme 2 are a combination of results obtained with the racemate and with the individual enantiomers of 1,3-butanediol. This example is one in which a marked "activation" of the enzyme is observed (Fig. 2). Thus the increase in rate observed at 0.25 M (*R*)-1,3-butanediol is nearly



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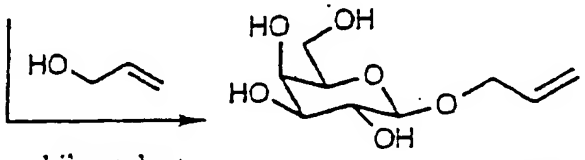
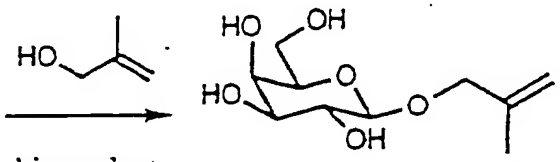


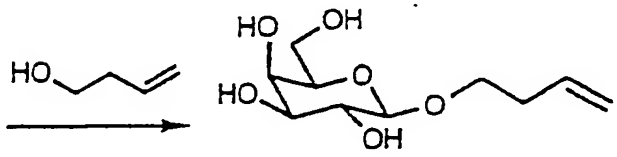
biocatalyst

20% yield[a]
5 yield[b]



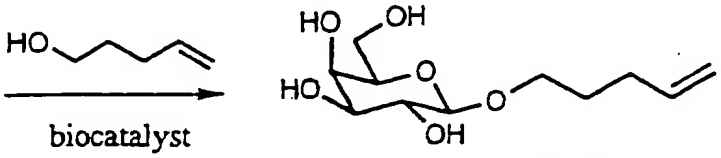
biocatalyst

12% yield[b]



biocatalyst

14% yield[b]



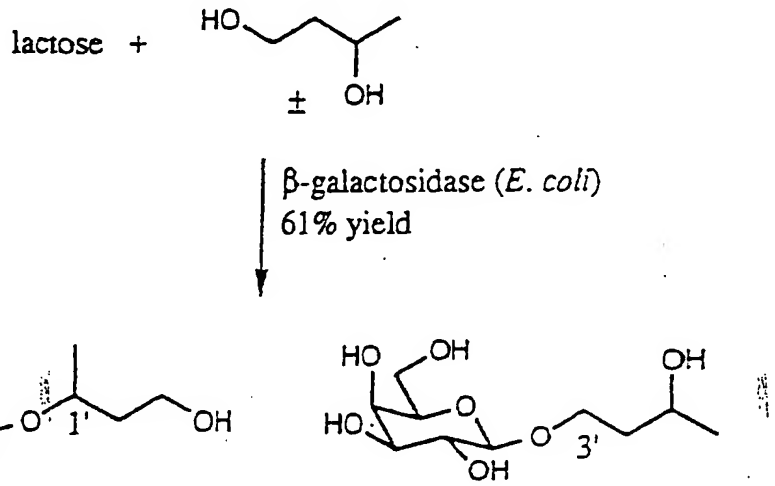
biocatalyst

4% yield[b]

[a]Biocatalyst: β-galactosidase from *Escherichia coli*

[b]Biocatalyt: β-galactosidase from *Aspergillus oryzae*

Scheme 1



Configuration at C-1'	Relative yield (%)	Configuration at C-3'	Relative yield (%)
R	100	*R*	100
S	50	*S*	99

Isomer	Relative transfer (Primary:secondary)
R	100:20
S	100:10

Scheme 2

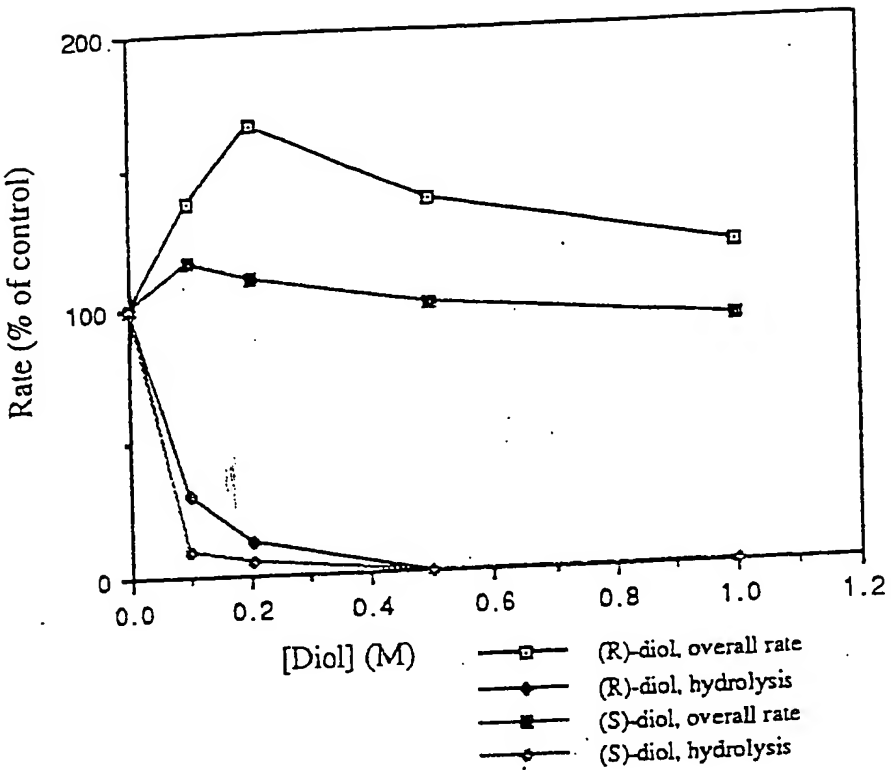
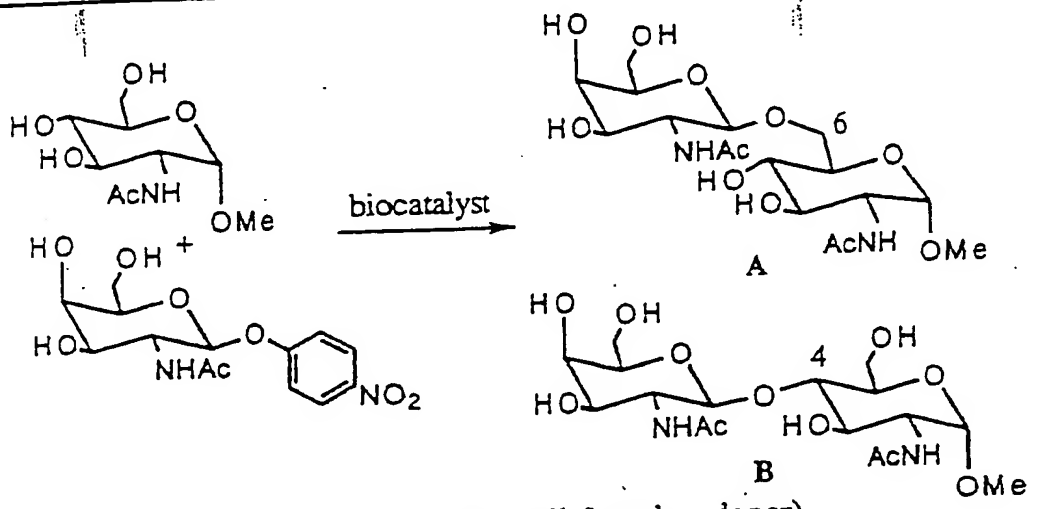


Figure 2. Overall rates of reaction and rates of hydrolysis of *o*-nitrophenyl-β-D-galactoside by the β-galactosidase of *Escherichia coli* in the presence of (*R*)- and (*S*)-1,3-butanediols.

170% of the rate of simple hydrolysis of the donor, *o*-nitrophenyl-β-D-galactoside under the same conditions. The relatively greater increase in rate with increasing (*R*)-enantiomer concentration relative to (*S*)-enantiomer is nicely paralleled by the selective transfer observed to the secondary hydroxyl group of the diol in preparative scale reactions using lactose as donor (Scheme 2). (Stereoselectivity is only expressed in transfer to the secondary position). It will be noted that the kinetic results (Fig. 2) indicate that at 0.5 M diol concentration, hydrolysis is reduced to zero and all of the glycosyl-enzyme intermediate is intercepted by diol.

In glycosidase-catalysed glycosyl transfer, the lack of regioselectivity observed in some cases is at the same time a drawback and an advantage. The drawback is that in preparative reactions a product separation step is necessary. The advantage is that

with sugar acceptors, transfer may be possible to more than one site in the acceptor. Most glycosidases used in these reactions are *exo*-glycosidases that operate only on the non-reducing terminal glycosidic bond in the oligosaccharide chain. For most of these enzymes, little is known about the contribution to the recognition process of non-terminal monosaccharide units. However, it has been found that even with simple aglycones, the configuration at the anomeric centre may significantly affect



A : B =1 : 9. Overall yield (A + B) =91% (based on donor)
Biocatalyst = β-*N*-acetylgalactosaminidase from *Aspergillus oryzae*

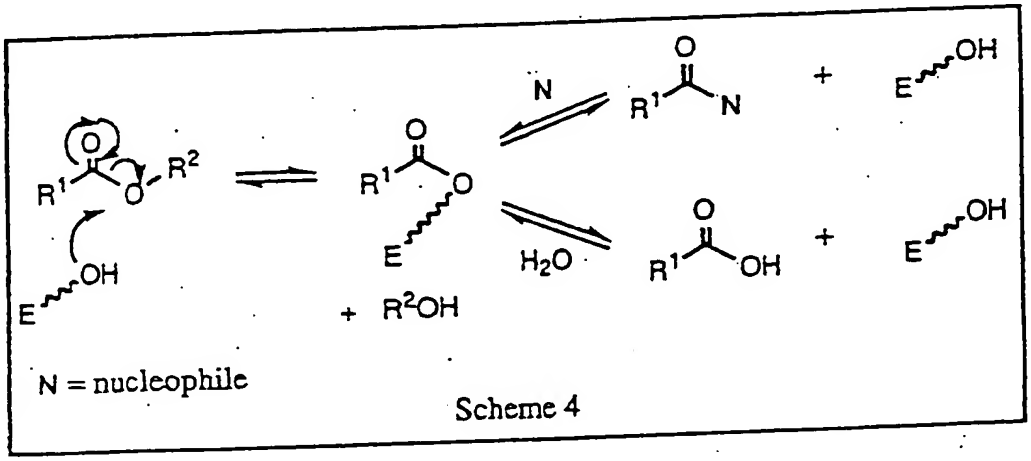
Scheme 3

regioselectivity. Thus in experiments with an *N*-acetylhexosaminidase from *Aspergillus oryzae*, it was found that transfer to C-4 of methyl α- and β-D-glucosides was favoured by an α-anomeric configuration (Crout *et al.*, 1991). Using this information, highly selective transfer to C-4 of methyl α-D-*N*-acetylglucosamine was achieved in the astonishingly high yield of 90% (Scheme 3) (Crout *et al.*, 1992). It is clear that with this enzyme there is very strong recognition at the acceptor binding site of hexoses with the glucose configuration. Given the plethora of glycosidases commercially available, it is clear that the glycosidase-catalysed approach to oligosaccharide synthesis has considerable potential. Investigations to date have barely progressed beyond the trisaccharide

stage, but as more is learnt about the recognition sites of these enzymes, it will be possible to apply them in a more rational and effective manner.

Protease-catalysed Peptide Synthesis

Enzymatic peptide synthesis has general features in common with glycosidase-catalysed glycosyl transfer. It has been widely applied to the practical synthesis of peptides (Kullman, 1984; Jakubke, Kuhl and Konnecke, 1985; Kasche, 1989) and may find a particular role in peptide manufacturing processes where the cost of production using conventional peptide reagents would be prohibitive. As with the glycosidase-catalysed approach, one of its attractions is the control of stereochemistry: problems of racemisation that attend conventional methods are largely, if not completely avoided. Indeed, the strict L-selectivity as far as the acyl donor is concerned provides an inbuilt discrimination mechanism, should any of the D-isomer be present. As with the glycosidase system, a problem with enzymatic peptide synthesis is that the products are always themselves substrates for the enzyme and can undergo hydrolytic degradation as they are formed. This problem can be minimised, as when using glycosidases, by using as acyl donors, *N*-protected amino acid esters rather than the less reactive amides. With enzymes such as α-chymotrypsin, this results in a shift of rate-determining step from acyl-enzyme formation to acyl-enzyme cleavage (Scheme 4). Also, the newly-created peptide, being much less reactive than the acyl donor, undergoes hydrolysis at a



Scheme 4

rate which is slow compared with its rate of formation. Even so, hydrolysis may occur, but can be minimised by carrying out the reaction in a medium of low water activity, in other words in an organic solvent of controlled water content. We have studied a system that was suggested by the observation (Dastoli, Musto and Price, 1966) that peptide hydrolysis can be catalysed by α-chymotrypsin suspended in nearly anhydrous dichloromethane. We argued that such a system might be used for peptide synthesis and this proved to be the case. Reactions were carried out in the system dichloromethane/0.25% water, in which the water content is just sufficient to saturate the organic solvent. Under these conditions product hydrolysis does not occur and high yields of peptides can be obtained (Ricca and Crout, 1989). In a brief survey, it is not possible to discuss all aspects of the results obtained, but those shown in Table 1 reveal some interesting features. Most striking is the observation that amino acid amides are much better nucleophiles than amino acid esters. This reflects the experience with aqueous systems, although the preference for amino acid amides rarely has been explicitly noted (Schellenberger and Jakubke, 1986).

Table 1. Peptide synthesis catalysed by α-chymotrypsin in dichloromethane

Donor ester	Nucleophile	Reaction product	Yield (%)	Time (h)
Ac-L-TyrOEt	L-PheNH$_2$	Ac-L-Tyr-L-PheNH$_2$	96	6
Ac-L-TyrOEt	L-LeuNH$_2$	Ac-L-Tyr-L-LeuNH$_2$	95	6
Ac-L-TyrOEt	L-LeuOMe	-	0	72
Ac-L-Tyr-OEt	L-ValNH$_2$	Ac-L-Tyr-L-ValNH$_2$	92	18
Ac-L-Tyr-OEt	L-AlaNH$_2$	Ac-L-Tyr-L-AlaNH$_2$	84	18
Ac-L-Tyr-OEt	L-MetNH$_2$	Ac-L-Tyr-L-MetNH$_2$	86	12
Ac-L-TyrOEt	L-ProNH$_2$	-	0	72
Ac-L-Tyr-OEt	L-LysOBu^i	Ac-L-Tyr-L-LysOBu^i	86	18
Z-L-TyrOEt	Gly-Gly-OEt	Z-L-Tyr-Gly-GlyOEt	88	18

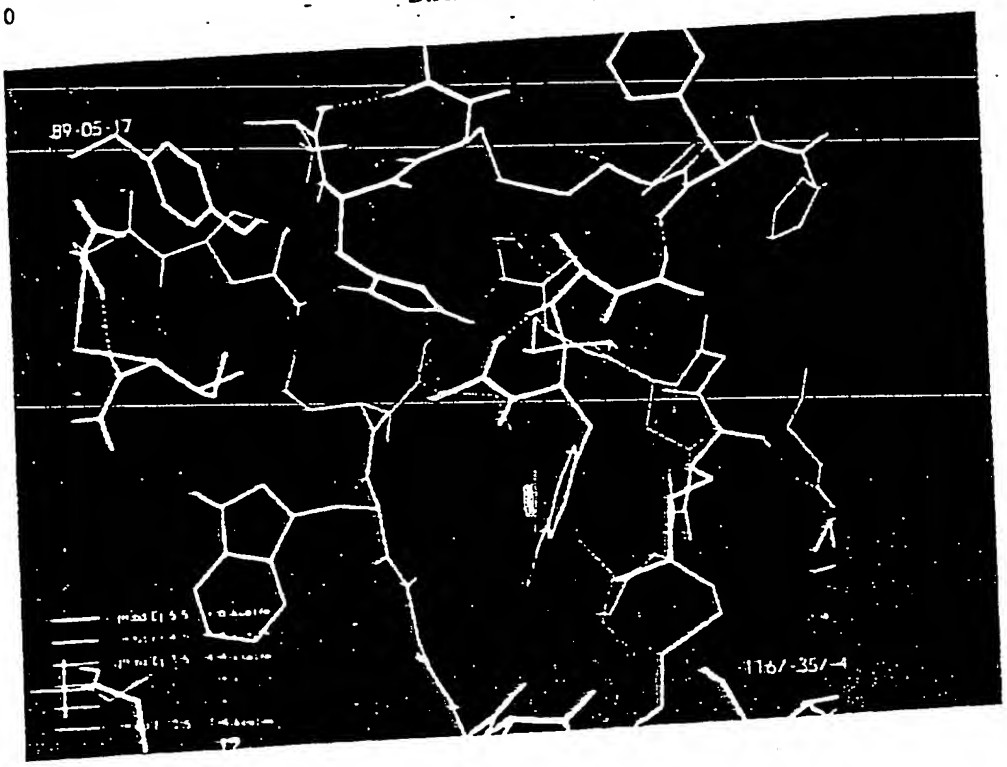


Figure 3. Molecular modelling of the oxyanion formed from *N*-acetyl-L-tyrosinyl-L-alaninemethylamide at the active site of α-chymotrypsin. (See Colour Plate 1).

Molecular modelling provided an explanation for this observation. Thus in Fig. 3 is shown the reult of energy minimisation of the oxyanion formed from *N*-acetyl-L-tyrosinyl-L-alaninemethylamide, which reveals the formation of a hydrogen bond between the amide amino group and the carbonyl oxygen atom of Phe41 (an interaction first proposed on the basis of a mechanical model (Bizzozero *et al.*, 1982). This strongly suggests that it is the loss of this hydrogen bond when the amide is changed to an ester group that results in a lower reactivity of amino acid esters compared with amino acid amides. In aqueous systems, the contribution to catalysis of this hydrogen bond is offset by the loss of comparable hydrogen bonds to solvent water. However, in the organic solvent system, there is no compensating loss and the full effect of the formation of the hydrogen bond can be expressed. By

contrast, the partitioning of the more hydrophobic ester between solvent and the enzyme-bound form will favour the dissolved state more than in the aqueous system. Thus differentiation between amino acid amides and esters would be expected to increase in the organic solvent system. Indeed, the entries for

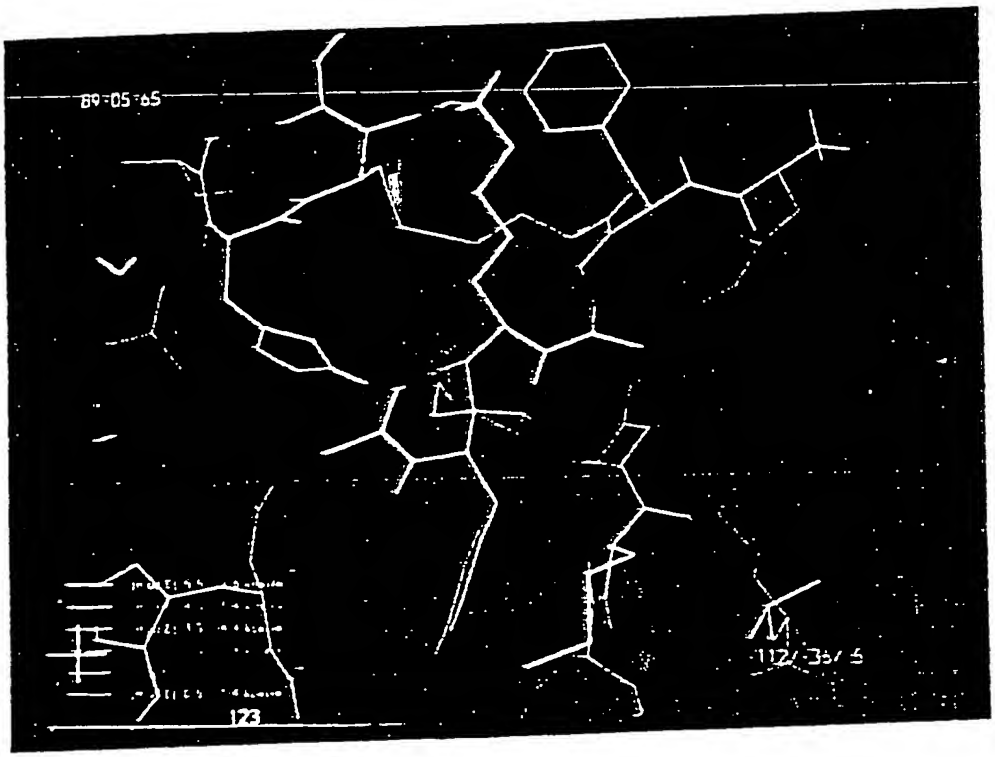
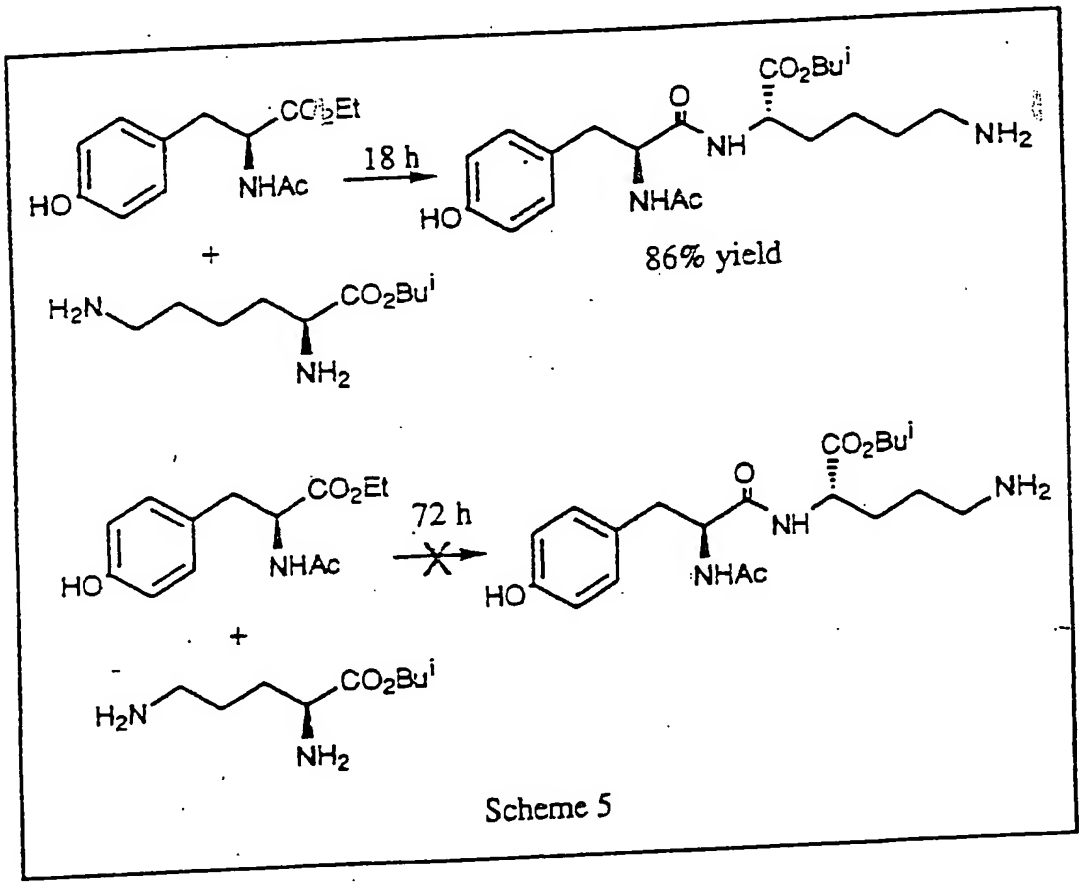


Figure 4. Molecular modelling of the oxyanion formed from *N*-acetyl-L-tyrosinyl-L-lysinemethylamide at the active site of α-chymotrypsin. (See Colour Plate 2).

L-LeuNH$_2$ and L-LeuOMe in Table 1 show that the difference becomes almost absolute. The analysis just presented might appear to be contradicted for the result for L-lysine *iso*-butyl ester (Table 1) which reacted as readily as most amino acid amides. Here again, molecular modelling provided an explanation. A corresponding oxyanion intermediate, when energy minimised, revealed that the side-chain could adopt a strainless antiperiplanar conformation in an orientation that

allowed formation of a hydrogen bond between the amino group and the backbone carbonyl group of Cys58 (Fig. 4). The validity of this interpretation was supported by subsequent studies of the corresponding reaction with the lower homologue, L-ornithine *iso*-butyl ester, which, having a side arm too short to allow generation of this hydrogen bond, was predicted to show a much lower reactivity. In the event, L-ornithine *iso*-butyl ester proved to be totally unreactive in this system (Scheme 5).



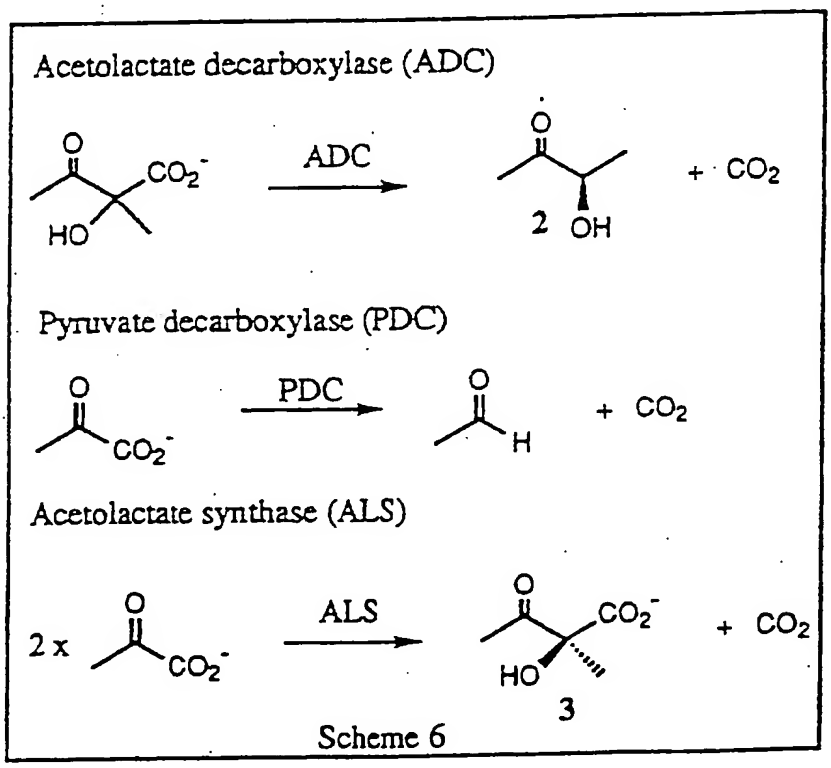
Scheme 5

These studies suggest that there is much to be gained in improving the selectivity and efficiency of biocatalytic processes from combining preparative, mechanistic and molecular modelling approaches. The rate at which X-ray crystallographic structures of important biocatalytic enzymes are becoming available will make possible further progress in this area.

APPLICATIONS OF DECARBOXYLATING ENZYMES

Transformations in which carbon-carbon bonds are produced are relatively rare in the biotransformation literature. We have studied three enzymes that catalyse carbon-carbon bond formation:

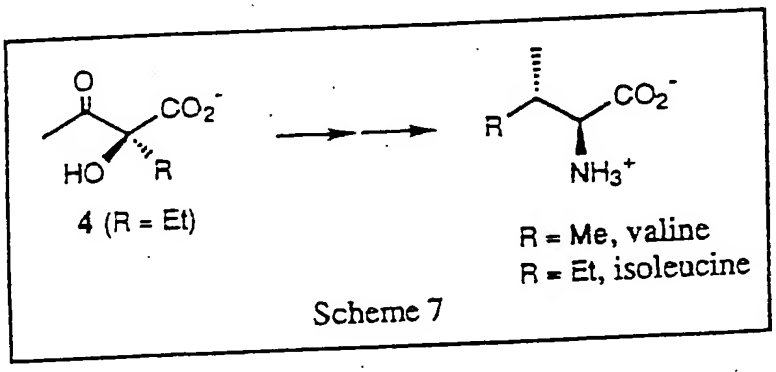
acetolactate decarboxylase (EC 4.1.1.5)
pyruvate decarboxylase (EC 4.1.1.1)
acetolactate synthase (EC 4.1.3.18).



Scheme 6

The use of the first two enzymes is unusual in that their normal function is to catalyse carbon-carbon bond cleavage (Scheme 6). However, both catalyse reactions in which carbon-carbon bonds are created. Pyruvate decarboxylase (PDC) catalyses intermolecular carbon-carbon bond formation whereas acetolactate decarboxylase, as will be shown below, catalyses an "abnormal" intramolecular carbon-carbon bond formation. Acetolactate decarboxylase (ADC), in its normal mode of action, catalyses the decarboxylation of α-acetolactate (2-hydroxy-2-

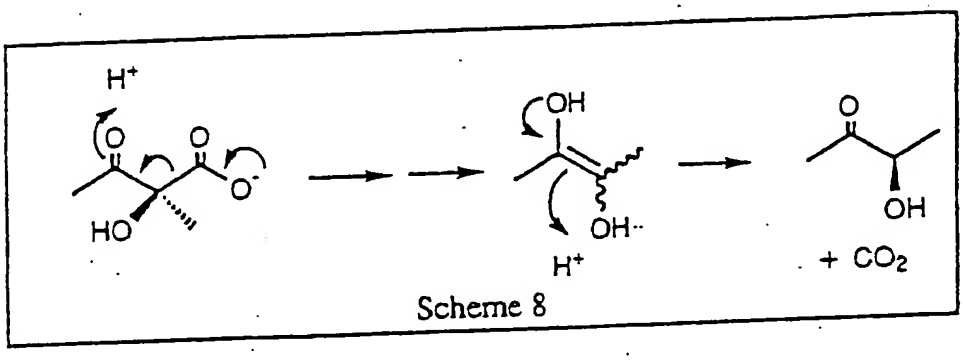
methyl-3-oxobutanoate) into (*R*)-acetoin ((*R*)-3-hydroxy-2-butanone) (Scheme 6). Acetolactate synthase is included, not because it is a synthetically useful enzyme (it has rather a narrow substrate range), but because its mode of action sheds light on the catalytic mechanism of pyruvate decarboxylase when it operates in the synthetic, as opposed to the normal degradative mode. (*S*)-α-Acetolactate 3 (Scheme 6) is a key intermediate in valine biosynthesis. Its higher homologue (*S*)-α-acetohydroxybutyrate ((*S*)-2-ethyl-2-hydroxy-3-oxobutanoate) (4 , Scheme 7) is the biosynthetic precursor of isoleucine, into which it is transformed by enzymes identical with those that catalyse the conversion of α-acetolactate into valine (Scheme 7) (Barak, Chipman and Schloss, 1988).



Scheme 7

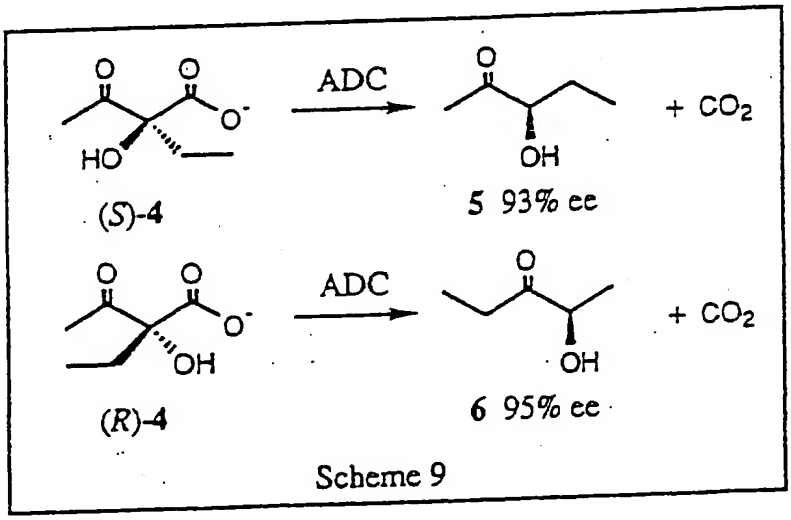
Biotransformations with Acetolactate Decarboxylase

Studies on the mechanism of acetolactate decarboxylase indicated that it reacted selectively with the (S)-enantiomer of α-acetolactate and that protonation of the presumed enediol intermediate occurred at the carbon atom that originally carried the carboxylate group, with overall inversion at the chiral centre (Scheme 8) (Hill, Sawada and Arfin, 1979; Crout *et al.*, 1984).However, when the decarboxylation of the higher homologue, α-acetohydroxybutyrate was examined, it was found that the reaction took place in two kinetically discrete stages. In the initial, faster reaction, the (*S*)-enantiomer was converted into (*R*)-3-hydroxy-2-pentanone 5.



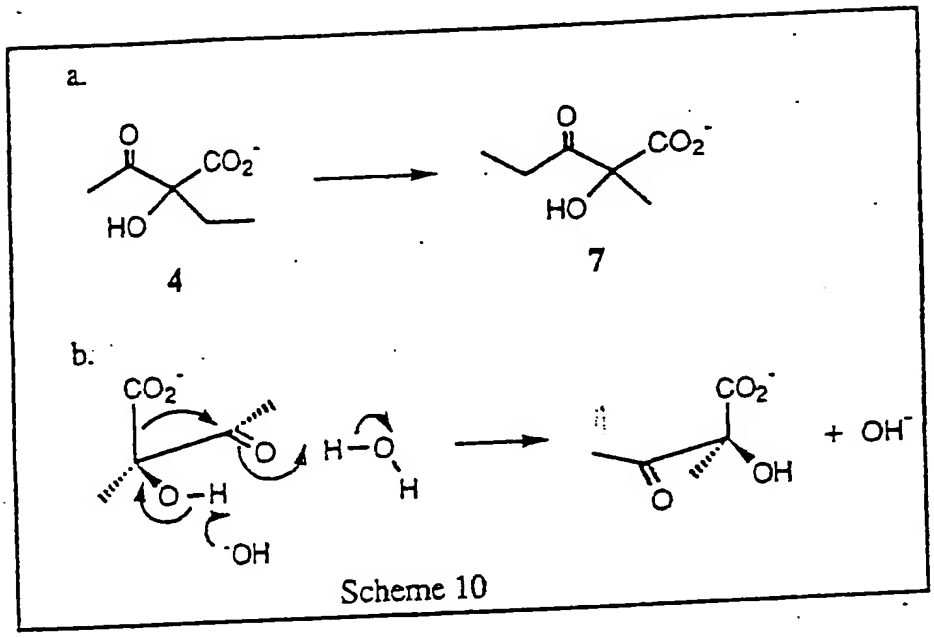
Scheme 8

However, this reaction was followed by a slower reaction in which the (*R*)-enantiomer was converted into (*R*)-2-hydroxy-3-pentanone **6**. Both ketol products were of high optical purity (Scheme 9) (Crout and Rathbone, 1988).

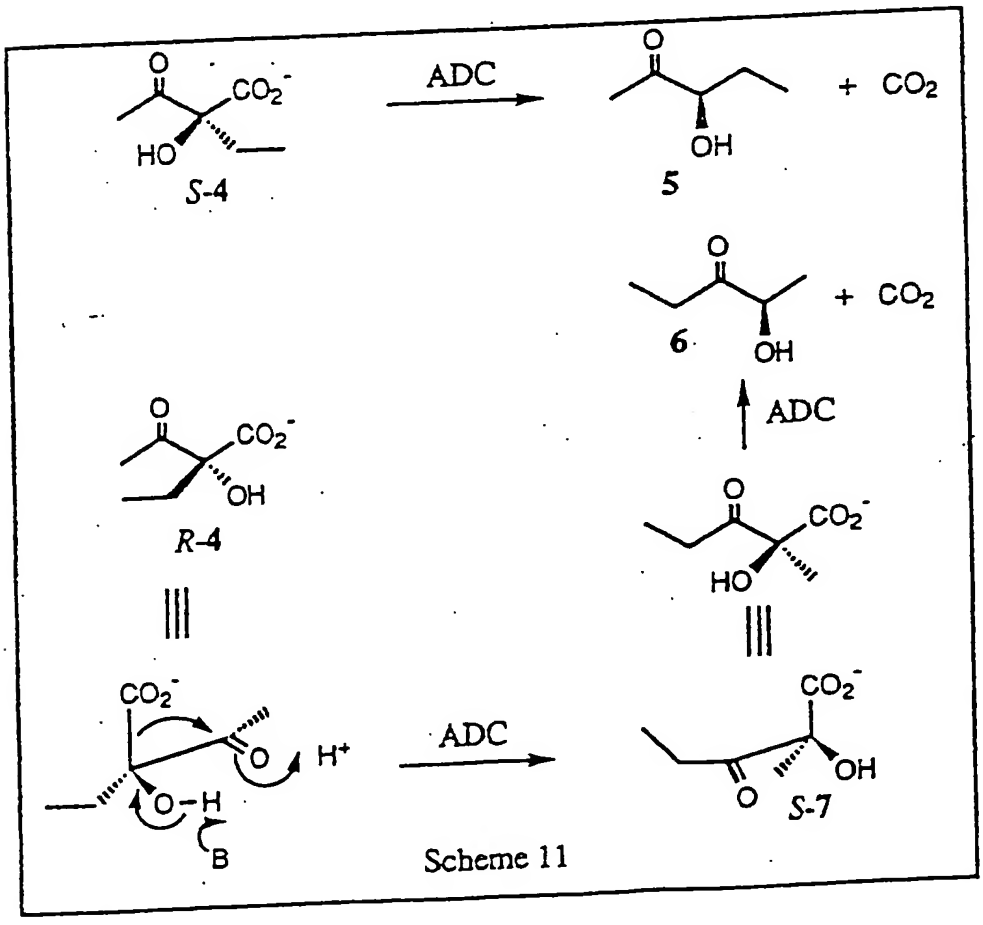


Scheme 9

We had previously observed that α-acetohydroxybutyrate underwent tertiary ketol rearrangement at pH >12.5 to the isomeric 2-hydroxy-3–oxocarboxylate **7** (Scheme 10a) (Crout *et al.*, 1984). This rearrangement was shown to proceed by intramolecular carboxylate ion migration using specifically ^{13}C-labelled α-acetolactate (Scheme 10b) (Crout and Hedgecock, 1979). It appeared possible that acetolactate decarboxylase was capable of catalysing a similar rearrangement. However, a dramatic catalytic effect would clearly be operating since ADC, which has an optimum at pH 6.5 for the "normal" reaction, is also able to catalyse this

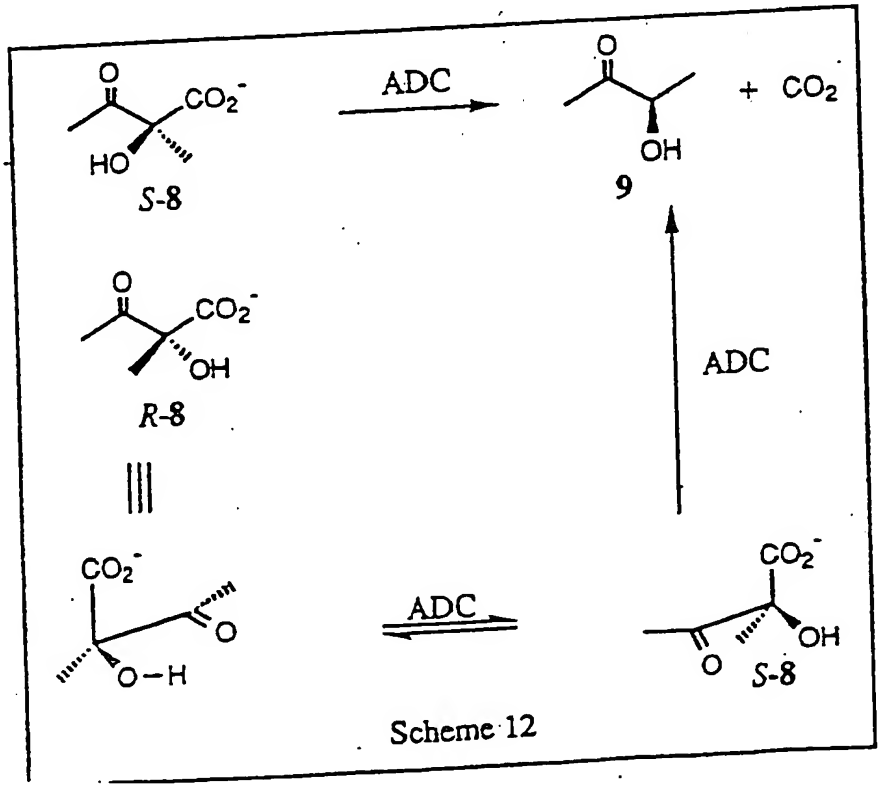


Scheme 10



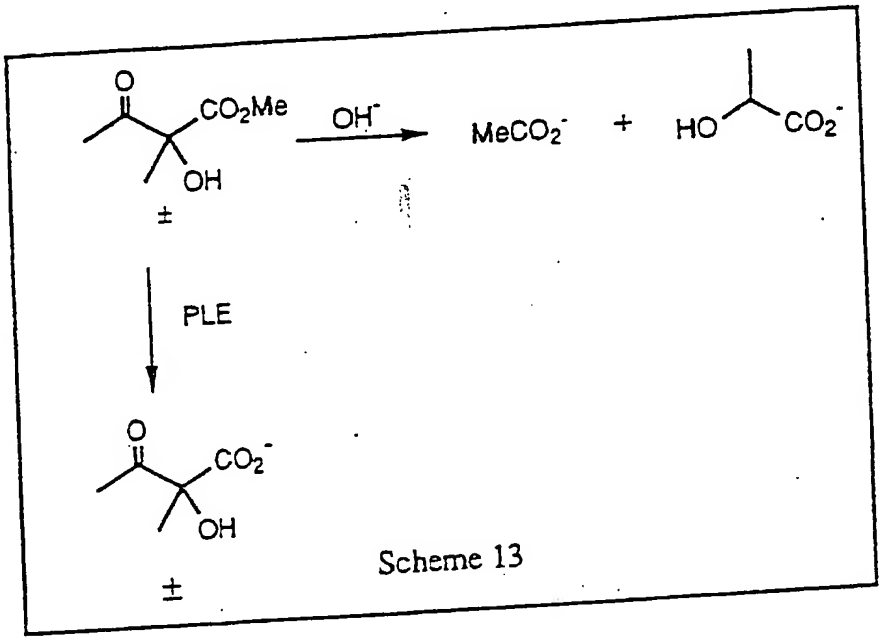
Scheme 11

rearrangement at this pH. Since the alkali-catalysed reaction occurs only at pH >12.5, a catalytic acceleration of $\sim 10^6$ is implied. Nevertheless, these observations suggested the interpretation shown in Scheme 11. It was postulated that S-4 underwent normal decarboxylation with overall inversion to give ketol 5. The enantiomer, *R*-4 underwent rearrangement with carboxylate migration to (*S*)-2-hydroxy-2-methyl-3-oxopentanoate, *S*-7. This now has the correct absolute configuration for decarboxylation to give ketol 6, isomeric with the "normal" product 5. If this proposal were correct, it suggested an intriguing possibility. Thus if the reaction were to be carried out with α-acetolactate, the rearrangement corresponding to *R*-4 → *S*–7 (Scheme 11) would be degenerate and the products of both the normal and the "abnormal" reactions would be identical (Scheme 12). The prediction therefore was that racemic α-acetolactate (±)-8 (Scheme 12) would be converted completely into (*R*)-acetoin 9. In putting this prediction to the test, a difficulty arising from a seemingly trivial operation had to be overcome, namely the hydrolysis of methyl α-acetolactate, the lability of which, and of analogous β-keto esters, requires their preparation from the corresponding esters immediately before

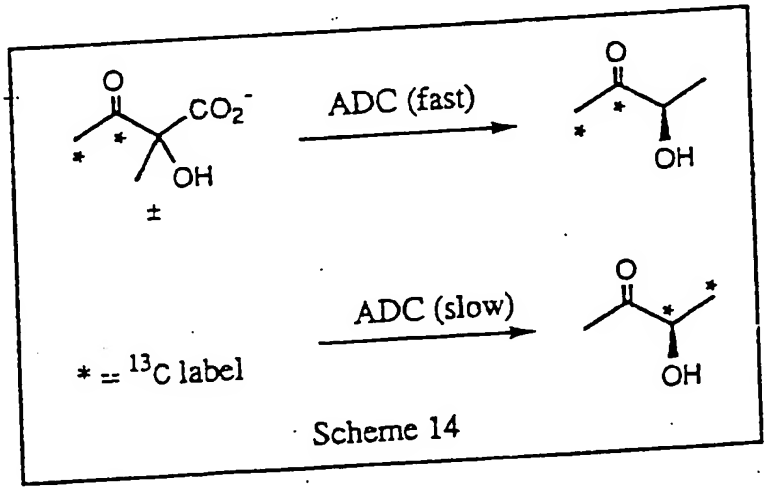


Scheme 12

any attempted enzymatic transformation. Alkaline hydrolysis always gives rise to a substantial amount of β-keto ester cleavage (Scheme 13). However, it was found that this difficulty was readily circumvented by subjecting the racemic methyl ester to the action of porcine liver esterase (PLE) which readily hydrolysed both enantiomers with little or no stereochemical discrimination (Scheme 13).



Scheme 13



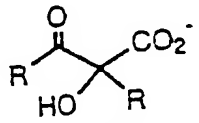
Scheme 14

When this reaction was carried out in the presence of acetolactate decarboxylase, (R)-acetoin (9, Scheme 12) of >98% ee was produced in quantitative yield (Crout

and Rathbone, 1988). Confirmation of the proposed mechanism (Scheme 12) was obtained by carrying out the reaction with doubly ^{13}C-labelled racemic α-acetolactate (Scheme 14). The expected isotopomer was formed during the first half of the reaction and the alternative isotopomer in a discrete second phase (Crout, Lee and Rathbone, 1990).

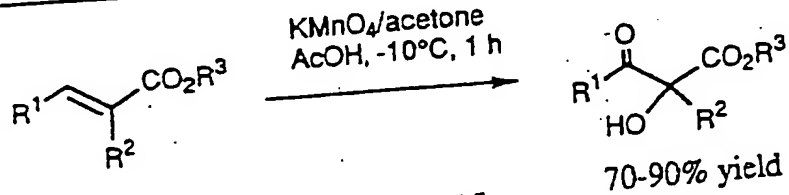
It should be noted that the degenerate rearrangement *R*-**8** to *S*-**8** (Scheme 12) is accompanied by overall inversion only when there is a *syn* arrangement of the oxygen substituents in the transition state. An *anti* arrangement would give rise to degenerate rearrangement but with overall retention.

The degeneracy of the rearrangement of Scheme 12 would be displayed by any substrate analogue of structure **10**, including cases in which R,R = $-(CH_2)_n-$.



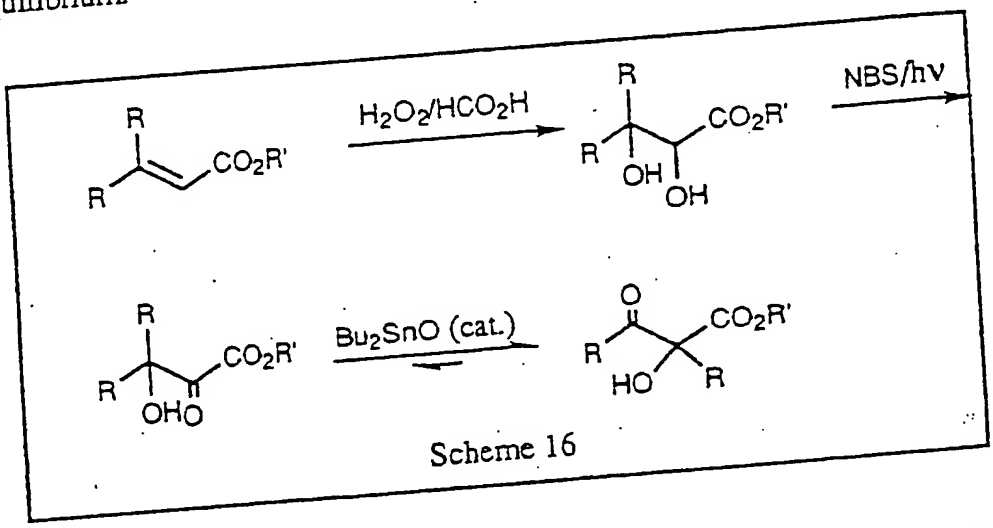
10

The substrate range of ADC was tested by synthesis of a range of α-acetolactate analogues with the symmetry expressed in structure **10**. Various routes to α-acetolactate analogues were used but the simplest and most direct was the four-electron permanganate oxidation of readily prepared (Wadsworth-Emmons) corresponding αβ-unsaturated esters (Scheme 15) (Crout and Rathbone, 1987).



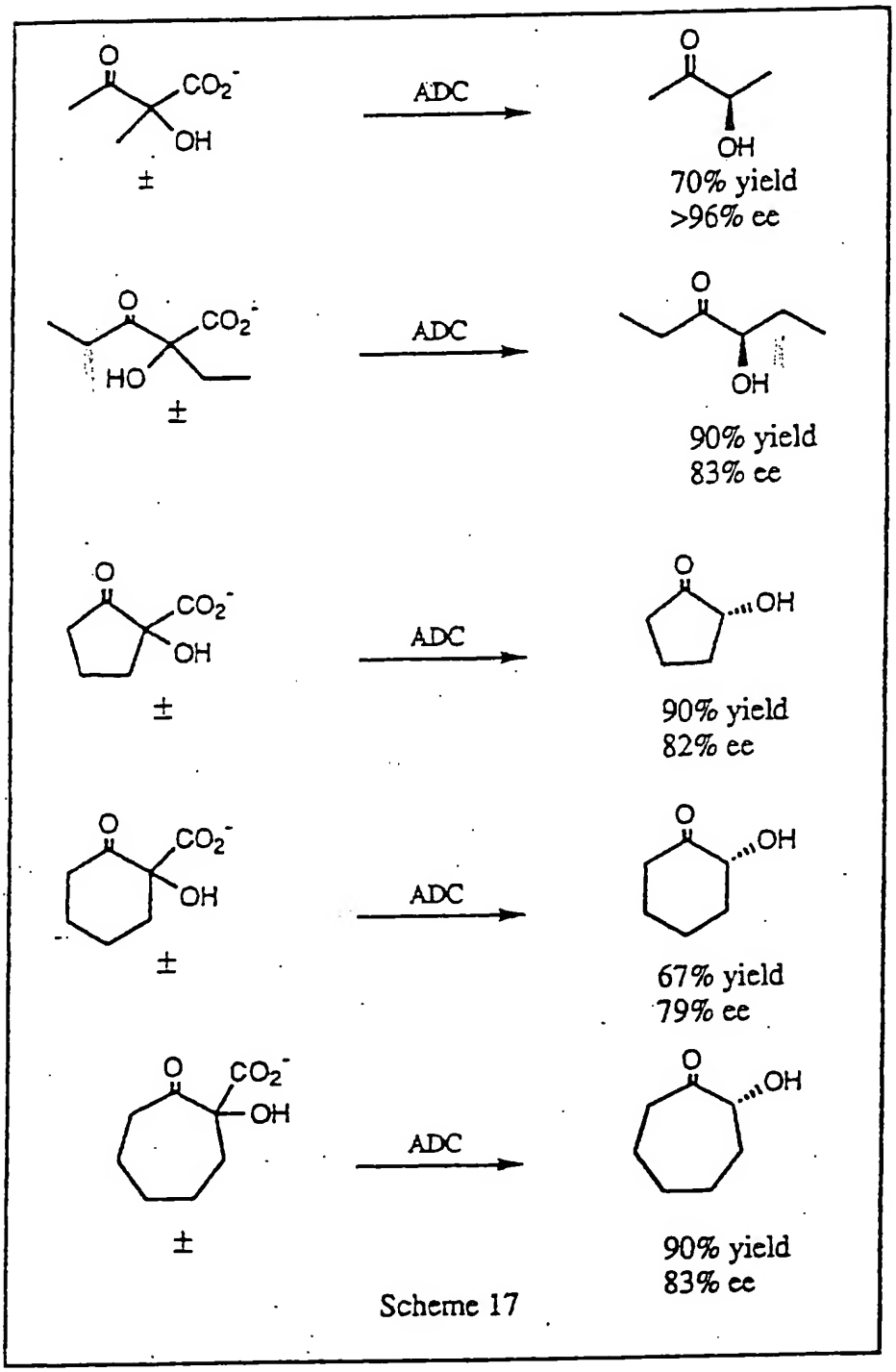
Scheme 15

A further intriguing method was based on the observation that with catalytic amounts of dibutyl tin oxide in boiling toluene, esters of 3,3-disubstituted 3-hydroxy-2-oxocarboxylates underwent tertiary ketol rearrangement quantitatively or nearly quantitatively into the corresponding α-acetolactate analogues (Scheme 16) (Crout and Rathbone, 1987). The rearrangement is reversible, but the 2-hydroxy-3-oxo esters appear to be thermodynamically more stable than the corresponding 3-hydroxy-2-oxo esters to the extent that little or none of the latter isomers are present at equilibrium.



Scheme 16

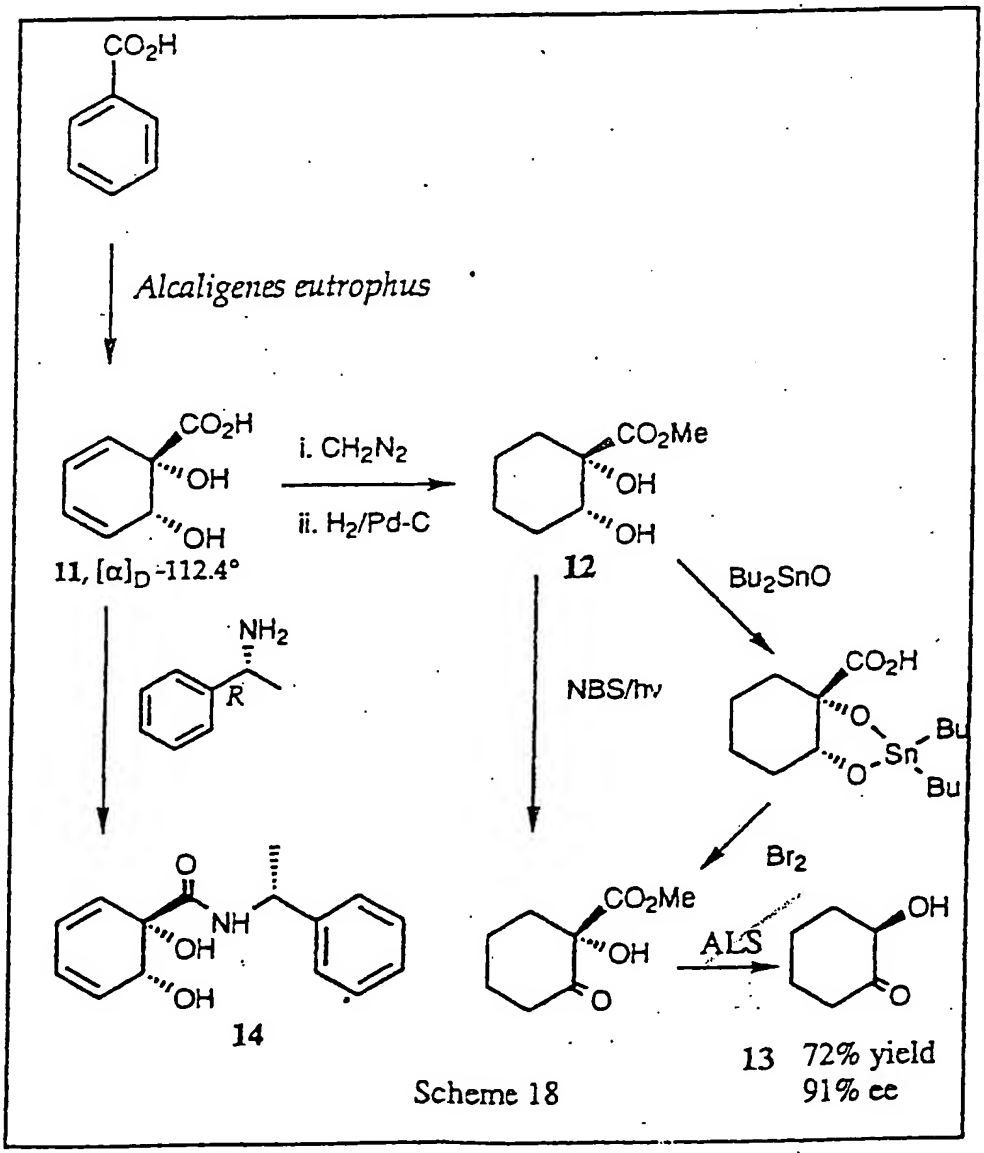
This route is intriguing because the rearrangement step mimics the corresponding rearrangement catalysed by the enzyme reductoisomerase during valine and isoleucine biosynthesis. Using the above methods, a series of α-acetolactate analogues were prepared and transformed by ADC to the corresponding ketols of high optical purity (Scheme 17), demonstrating that for all of these substrates, both the normal and the rearrangement pathway were operating to give essentially a single enantiomer of the product ketol. In all cases, conversion was continued until all of the substrate had disappeared.



Scheme 17

An interesting source of the cyclohexane analogue in optically pure form was the so-called benzoate diol 11 obtained by dihydroxylation of benzoic acid using

Alcaligenes eutrophus. This was converted into the α-acetolactate analogue **12** which in turn was readily converted by ALS into (*R*)-2-hydroxycyclohexanone **13** of high optical purity (Scheme 18). The greater reactivity of the analogue compared with the overall reactivity of the racemic material (Scheme 17) and its conversion into a ketol of higher optical purity suggested that it had the (1*S*, 2*R*)-configuration shown. This was confirmed by X-ray crystallographic analysis of the amide prepared from (*R*)-1-phenylethylamine **14** (Scheme 18, Fig. 5).



Scheme 18

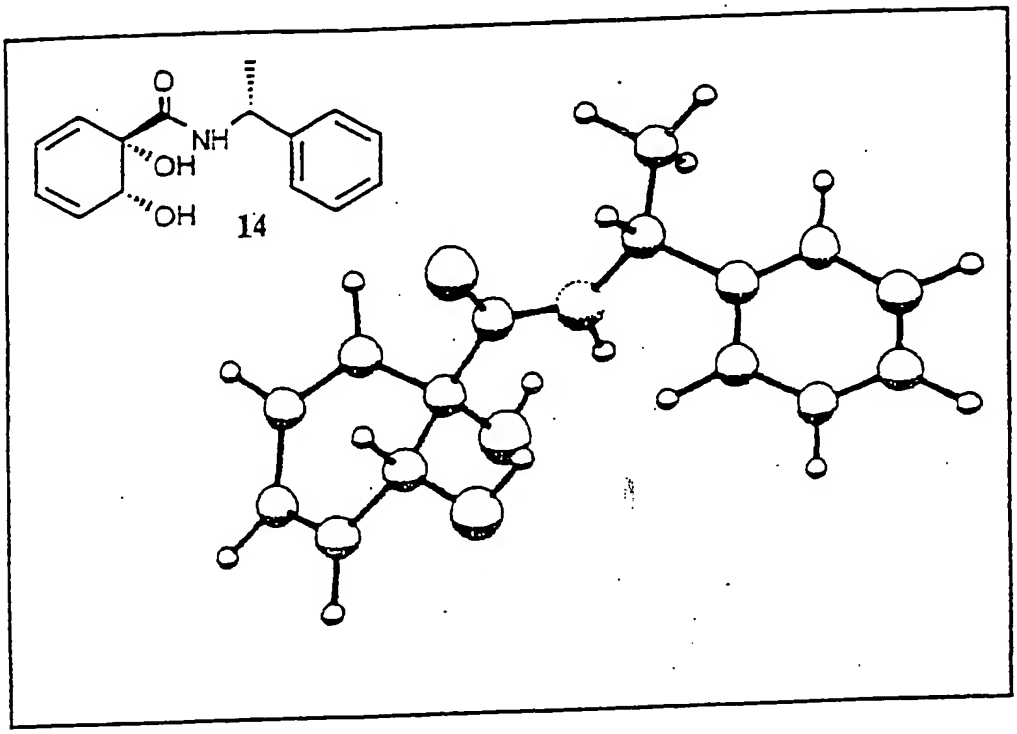
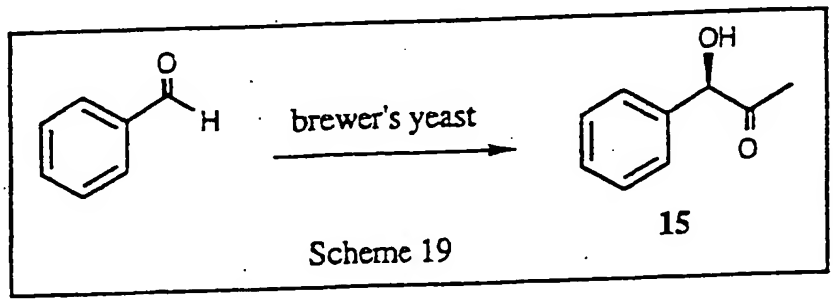


Figure 5. X-ray crystal structure of the amide **14**.

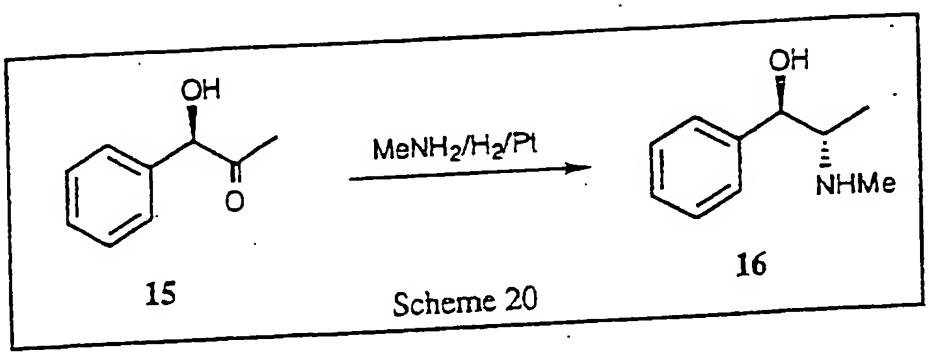
Biotransformations with Pyruvate Decarboxylase

Interest in pyruvate decarboxylase stemmed from its possible involvment in a reaction (Scheme 19) first reported in 1921 (Neuberg and Hirsch, 1921). Benzaldeyde, in fermenting brewer's yeast, was converted into 1-phenyl-1-hydroxy-2-propanone (phenyl acetyl carbinol) **15**.



Scheme 19

This transformation assumed economic importance when it was incorporated into a process for the production of ephedrine 16 (German Patent, 1932). Subsequently a

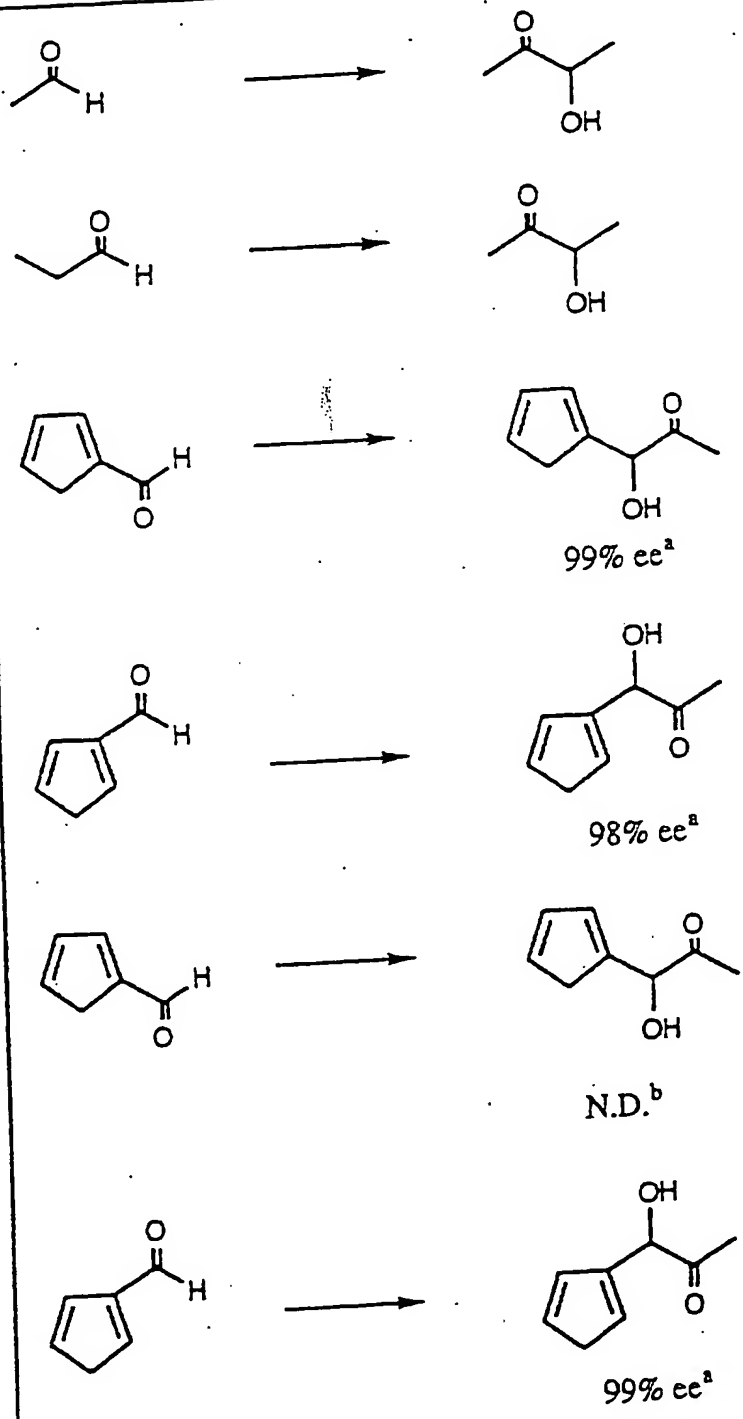


15 16

Scheme 20

wide range of aldehydes was shown to be converted into acyloins in this system (e.g. Schmauder and Gröger, 1968; Bernardi *et al.*, 1980; Fuganti *et al.*, 1984; Long, James and Ward, 1988). Although pyruvate decarboxylase had been suggested as the enzyme responsible for phenyl acetyl carbinol production (Hanc and Kakac, 1956; Voijtisek and Netrval, 1982) it was only recently that definitive studies (Bringer-Meyer and Sahm, 1988) demonstrated the conversion of benzaldehyde into phenyl acetyl carbinol by the pyruvate decarboxylases of *Saccharomyces carlsbergensis* and *Zymomonas mobilis*. Meanwhile, Juni had shown that acetoin formation from pyruvate and acetaldehyde was also catalysed by pyruvate decarboxylase (Juni, 1961). Juni showed that at sufficiently high acetaldehyde concentrations, pyruvate decarboxylation was diverted entirely to acetoin production.

We were interested in confirming the role of pyruvate decarboxylase in these transformations and exploring the usefulness of the enzyme in biotransformations. Pyruvate decarboxylase from yeast (Sigma) and from *Zymomonas mobilis* (cloned and expressed in *Escherichia coli*) were used in these studies. In Scheme 21 is shown a range of aliphatic and heterocyclic aldehydes that were found to be converted into acyloins by yeast PDC in the presence of pyruvate.

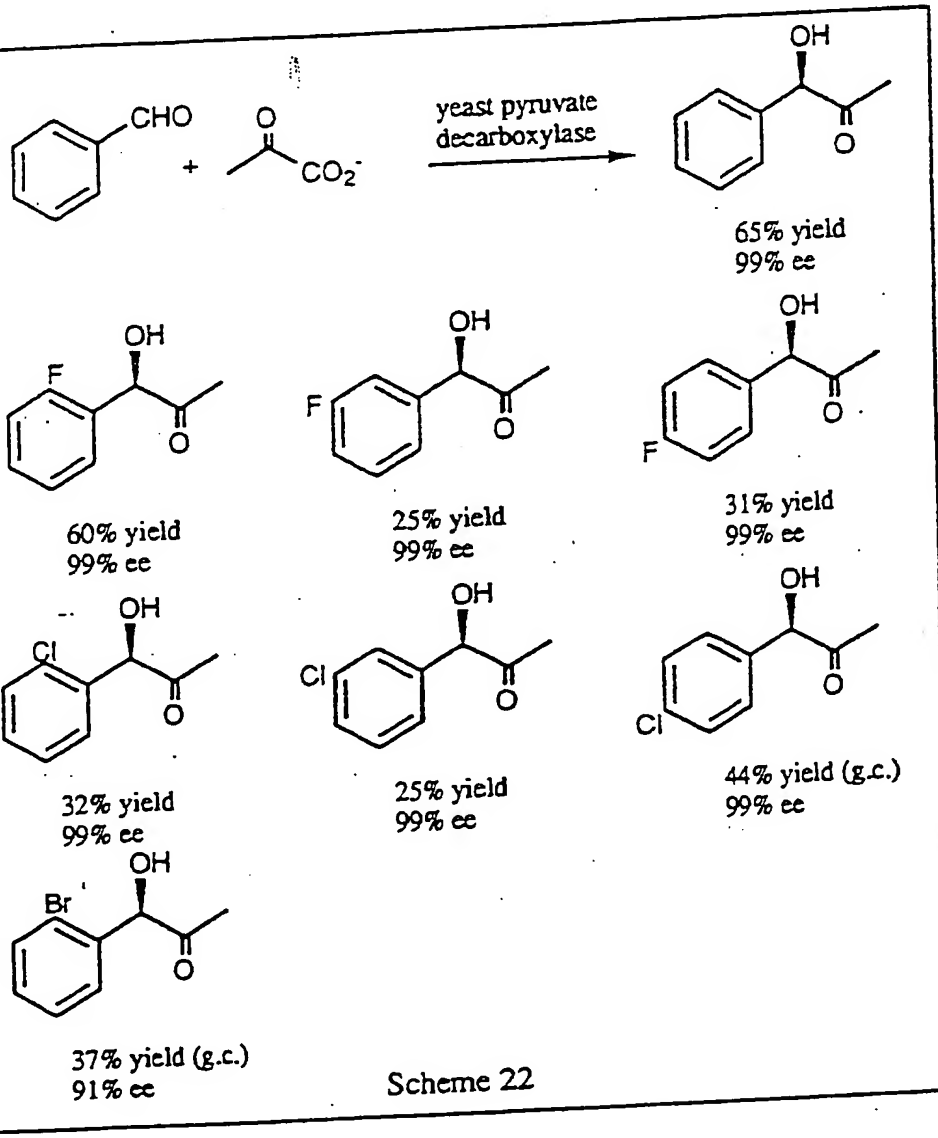


[a]Absolute configuration not determined

[b]N.D.: not determined.

Scheme 21

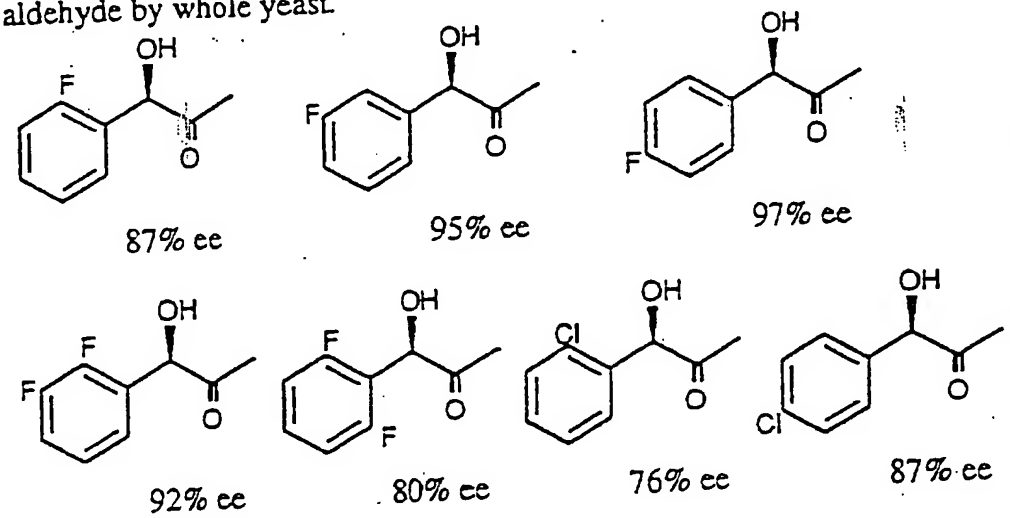
The situation with regard to acetoin is unusual. Yeast PDC and *Z. mobilis* PDC each catalysed decarboxylation to give acetoin of intermediate and reproducible optical purity, but of opposite configuration. The significance of this finding has been discussed (Bornemann *et al.*, 1993). Studies with benzaldehyde and a range of substituted benzaldehydes gave corresponding acyloins of high optical purity (Scheme 22). The acyloins were shown by circular dichroism uniformly to have the *R*-configuration (Kren *et al.*, 1993). Acyloins obtained using whole yeast were



Scheme 22

shown to have the same absolute configurations. However, these products were isolated with consistently lower optical purities than those obtained using the isolated enzyme (Scheme 23). It was of interest that the fluorinated benzaldehydes were all better substrates than benzaldehyde (relative initial rates 100:162:136:110 for benzaldehyde, *o*-, *m*- and *p*-fluorobenzaldehyde).

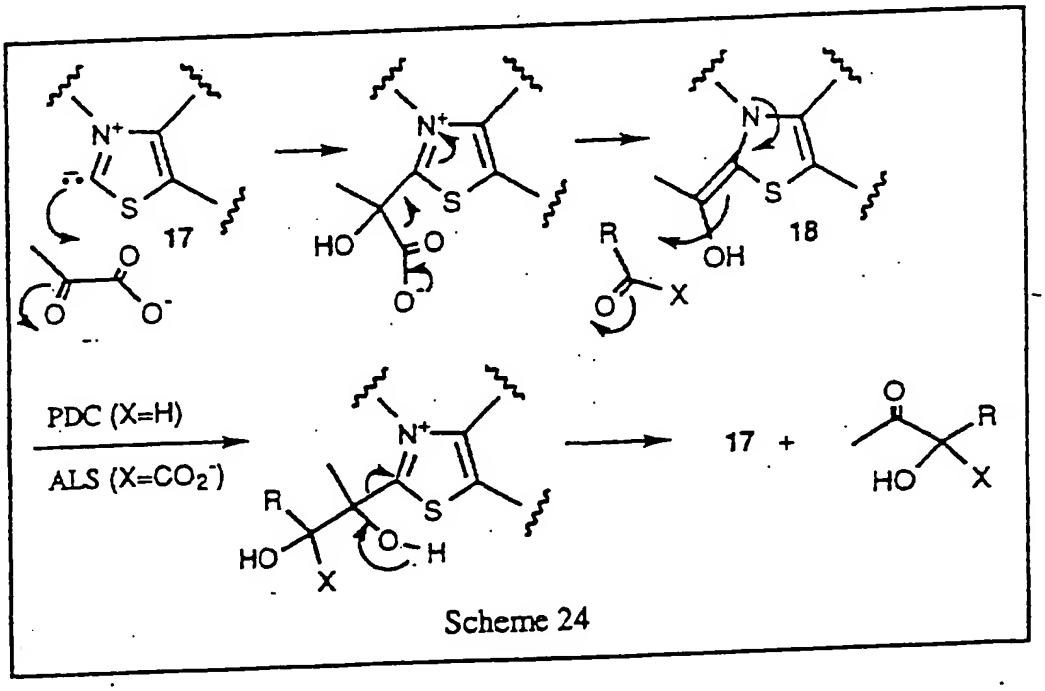
Optical purities of acyloins produced from the corresponding aldehyde by whole yeast.



Scheme 23

These studies conclusively demonstrate that pyruvate decarboxylase is the enzyme responsible for acyloin production in fermenting yeast (*Saccharomyces* sp.). This is shown by the production of a range of acyloins also produced by fermenting yeast and by the identity of the absolute configurations of the products obtained using the isolated enzyme and the whole cell system. Potentially, PDC is an enzyme useful for the production of acyloins because they are produced with a higher optical purity than in the whole cell system. In addition, use of the enzyme avoids the problem, inherent in the whole-cell system, of diversion of much of the aldehyde to the corresponding alcohol through the action of dehydrogenases.

A major question remains. Why should pyruvate decarboxylase, a catabolic enzyme (Scheme 6), catalyse carbon-carbon bond formation? A clue is provided by the recent discovery (Green, 1989) that pyruvate decarboxylase is homologous with acetolactate synthase (Scheme 6). Both are thiamin pyrophosphate-dependent enzymes in which the intermediate **18** (Scheme 24) is condensed either with an aldehyde (PDC) or with pyruvate (ALS) to give respectively an acyloin or α-acetolactate. PDC and ALS therefore clearly belong to a family of enzymes that includes pyruvate oxidase (Schloss and Aulabaugh, 1990). The ability of aldehydes to trap the intermediate **18** (Scheme 24) in PDC-catalysed reactions may be attributable to binding to a vestigial site that in pyruvate oxidase binds the quinone cofactor necessary for re-oxidation of FAD. The inhibition of ALS by herbicides is attributed to binding to a similar vestigial quinone binding site, blocking the binding site for the second α-keto acid substrate (Schloss and Aulabaugh, 1990).



Scheme 24

Acknowledgements

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